CELLULOSE PHOSPHATE FROM OIL PALM BIOMASS AS POTENTIAL BIOMATERIAL

BY

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LIST OF ABBREVIATES

CP	Cellulose phosphate
DP	Dissolving pulp
DS	Degree of substitutions
EFB	Empty Fruit Bunch
Et_3PO_4	Tri-ethyl phosphate
H_3PO_4	Phosphoric acid
IC_{30}	70% cell growth
IC_{50}	50% cell inhibited
L929	Mouse Skin fibroblast cell line
MCC	Microcrystalline cellulose
OD	Optical density
-OH	Hydroxyl group
OPEFB	Oil Palm Empty Fruit Bunch
OPEFB-MCC	Microcrystalline cellulose from Oil Palm Empty Fruit Bunch
OPEFB-CP	Cellulose phosphate from Oil Palm Empty Fruit Bunch
P	Phosphorus contains
P_2O_5	Phosphorus Pentoxide
$-PO_4$	Phosphate group
SBF	Simulated Body Fluid

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ABSTRACT

OPEFB-CP with a DS of 2.4 was synthesized from oil palm biomass via the phosphorylation of microcrystalline cellulose (OPEFB-MCC) using the H_3PO_4/ P_2O_5/ Et_3PO_4/ hexanol method. Characterization of OPEFB-CP was performed using scanning electron microscopy (SEM), energy dispersive analysis (EDX), Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), Thermogravimetry (TG) and water absorption. The biocompatibility in terms of cytotoxicity of OPEFB-CP was performed on mouse skin fibroblast cells (L929) by MTS Assay analysis and Trypan Blue Exclusion by indirect extraction method. The proliferation rate of L929 cell on OPEFB-CP IC_{50} concentration was accessed up to 8 days. The bioactivity of OPEFB-CP was studied by immersion of OPEFB-CP in Simulated Body Fluid (SBF) for 30 days in order to observe its applicability to form apatite nuclei.

Results showed that phosphorylation has changed the OPEFB-MCC surface morphology, where OPEFB-CP has a more sponge-like surface character and compact structure. EDX confirmed the presence of phosphorous with a 16.4% increase in atomic weight of P upon phosphorylation. The presence of new FTIR peaks at 2380 cm^{-1}, 1380 cm^{-1} and a shoulder at 920–1000 cm^{-1} are indications of a successful grafting of the phosphate groups on the cellulose backbone. XRD data revealed that most of the diffraction bands of OPEFB-MCC were depressed or absent demonstrating the loss of crystallinity of the material after phosphorylation. OPEFB-MCC have a higher thermal stability as compared to OPEFB-CP, nevertheless

OPEFB-MCC presents a total loss of 90% while the OPEFB-CP showed a loss of only 62%; the smaller rate of mass loss of the latter is attributed to some kind of thermal protection of the phosphate on the product. OPEFB-CP has also higher water absorption capacity.

OPEFB-CP showed non cytotoxicity reaction on L929 cells depending on the dilution concentration of extract used with an IC_{50} value of OPEFB-CP of 45 % after 72 hours of cell exposure. No change in cell morphology can be observed when the concentration used is below the IC_{50} concentration, however with concentrations higher than IC_{50}, the cells became swollen and lost their membrane integrity. After immersion in SBF for 30 days, apatite formation was formed on OPEFB-CP surfaces which was treated with Ca $(OH)_2$. However there is no sign of the apatite layer on untreated OPEFB-CP surface. The sharp peak at 874 cm^{-1} is indicative of the deposition of apatite layer on the surface of OPEFB-CP-Ca. SEM-EDX analysis confirmed the formation of apatite with Ca to P molar ratio of 1.85; a characteristic peak of apatite at 2θ= 26.10° further strengthened the evidence of apatite formation on OPEFB-CP-Ca. Results demonstrate that cellulose phosphate from oil palm lignocellulosic residual has the potentials to be applied in biomedical applications.

SELULOSA FOSFAT DARIPADA BIOJISIM KELAPA SAWIT SEBAGAI BIOBAHAN BERPOTENSI

ABSTRAK

OPEFB-CP dengan DS 2.4 telah disintesis daripada biojisim kelapa sawit melalui fosforilasi selulosa mikrohablur (OPEFB-MCC) dengan mengguna kaedah H_3PO_4 / P_2O_5 / Et_3PO_4 / heksanol. Pencirian bagi OPEFB-CP telah dilakukan menggunakan mikroskop pengimbas elektron (SEM), tenaga dispersif analisis (EDX), spektrofotometer transfomasi inframerah fourier (FTIR), X-ray difraksi (XRD), kalorimetri pengimbas perbezaan (DSC), Termogravimetri (TG) dan penyerapan air. Penilaian toksik bagi sample OPEFB-CP dijalankan dengan menggunakan sel fibroblas kulit tikus (L929), dan analisis esei MTS dan teknik perwarnaan trypan biru, berasaskan kaedah ekstraksi tidak langsung digunakan sebagai kaedah analysis. Kadar proliferasi L929 terhadap ekstrak OPEFB-CP dengan menggunakan kepekatan IC_{50} selama 8 hari juga dilakukan. Sifat bioaktif OPEFB-CP dikaji dengan merendam OPEFB-CP di dalam SBF selama 30 hari dalam usaha meneliti kebolehupayaan OPEFB membentuk pembentukan tulang.

Keputusan kajian menunjukkan fosforilasi telah mengubah morfologi permukaan OPEFB-MCC, di mana OPEFB-CP memiliki sifat permukaan berongga seperti span dan struktur yang lebih padat. Analisis EDX mengesahkan kehadiran fosforus dengan peningkatan 16.4% dalam berat atom P selepas fosforilasi. Kehadiran puncak FTIR baru di 2380 cm^{-1}, 1380 cm^{-1} dan bahu di 920-1000 cm^{-1} adalah penunjuk kejayaan pencantuman kumpulan fosfat pada tulang belakang selulosa. Analisis XRD menunjukkan fosforilasi mengubah struktur hablur OPEFB-MCC di mana sebahagian besarnya hilang dan mengalami kemelesetan. OPEFB-MCC mempunyai kestabilan terma yang lebih baik berbanding dengan OPEFB-CP,

namun OPEFB-MCC menunjukan 90% jumlah kehilanga berat akhir sedangkan OPEFB-CP menunjukkan kehilangan hanya 62 %; tahap kehilangan yang lebih kecil oleh OPEFB-CP dikaitkan dengan perlindungan terma daripada fosfat yang terdapat pada produk. OPEFB-CP menunjukkan sifat tahap penyerapan air yang lebih tinggi daripada OPEFB-MCC.

Sifat toksik OPEFB-CP ke atas sel L929 adalah bergantung pada pencairan kepekatan ekstrak yang digunakan dengan nilai IC_{50} OPEFB-CP adalah pada 45 % selepas 72 jam di dedahkan kepada sel. Tiada sebarang perubahan yang dapat di perhatikan ke atas morfologi sel apabila kepekatan digunakan di bawah kepekatan IC_{50}. Namun dengan kepekatan yang lebih tinggi daripada IC_{50}, sel-sel menjadi bengkak dan kehilangan integriti membran. Setelah direndam dalam SBF selama 30 hari, terdapat pembentukan apatit pada permukaan OPEFB-CP-Ca yang di rawat dengan Ca $(OH)_2$. Namun tidak ada tanda lapisan apatit pada permukaan OPEFB-CP yang tidak dirawat. Puncak di 874 cm^{-1} menunjuk pengendapan lapisan apatit pada permukaan OPEFB-CP-Ca. SEM-EDX analisis membuktikan pembentukkan apatit dengan nisbah molar Ca kepada P ialah 1.85; puncak apatit pada 2θ= 26.10° menguatkan lagi bukti pembentukkan apatit pada OPEFB-CP-Ca. Keputusan menunjukkan bahawa selulosa fosfat daripada kelapa sawit sisa lignoselulosa mempunyai potensi dalam aplikasi bioperubatan.

CHAPTER 1

INTRODUCTION

1.1 General

The growth of oil palm industry in Malaysia has been one phenomenal, and the country is now one of the largest producer and exporter of palm oil in the world. Malaysia is blessed with huge quantities of oil palm biomass, with more than 4.69 million hectares land under oil palm cultivation, and producing 17.73 million tonnes of palm oil annually (MPOB 2009). Due to this situation, enormous amounts of lignocelluloses residues from trunks, fronds and the empty fruit bunches (EFB) were generated from palm oil industry, with the latest estimate of more than 15 million tons (Abdul Khalil *et al.*,2007). Oil constitutes only about 10 % of the palm production while the rest is biomass which is available throughout the year as EFB and pruned fronds (Rozman *et al.*, 1998; Amin *et al.*, 2007).

Attempts have been made in turning these wastes into value added product, converting this lignocellulosic residue to paper-making pulp is an example. Nevertheless it is a relatively "low end" one, where the price per tonne is rather low (WanRosli *et al.*, 1998, 2007). Hence, other utilization alternatives are sought, one of which is conversion into cellulose derivatives, such as carboxymethylcellulose (CMC), cellulose acetate and cellulose phosphate (CP), and the latter is of significant importance due to its potentials to be used as biomaterials.

The term biomaterial refers to any materials, either natural or man-made that are intended to interface with biological systems or biomedical device for evaluating, treating, augmenting, or replacing any tissue, organ, or function of the body (Agrawal, 1998; Matthew, 2002; Nair & Laurencin, 2005). Biomaterial elements

encompass in medicine, biology, chemistry, tissue engineering and material science. Biocompatibility and bioactivity are the essential properties to qualify any material as a biomaterial (Entcheva *et al.,* 2004).

Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. From clinical perceptive these materials were not producing a toxic or injurious reaction and not causing immunological rejection (Matthew 2002; Vepari and Kaplan 2007; Correlo *et al.,* 2008). Bioactivity on the other hand refers to the capability of material to mineralize in physiological environment. In orthopaedic application bioactivity was referred to the ability of material to induce the formation of an apatite layer (calcium phosphate layer) in simulated plasma solutions (Granja *et al.,* 2001b, 2005).

Several classes of materials such as ceramics, metal, glasses, natural and synthetic polymeric materials have been investigated as biomaterials for medical use especially in tissue engineering and orthopedic applications (Burg, *et al.,* 2000, Nair & Laurencin 2005, Barbosa *et al.,* 2005; Correlo *et al.,* 2008). Some of these materials have drawbacks and limitation such as metallic implants used as bone repairing which will cause stress shielding and bone resorption due to the elasticity mismatch with the surrounding bone. Meanwhile ceramic materials are not favourable for bone repairing material because of its poor resistance against fatigue failure and low fracture toughness. Although modulus of elasticity of less rigid titanium is five times higher than human bone, it still has limitation for their extensive application in bone reconstruction (Li *et al.,* 1997; Nair & Laurencin 2005).

Due to socioeconomic situation of the modern world and environmental concerns, interests in using natural biodegradable polymer are on the rise. These are due to their biodegradability, low toxicity and low disposal costs that make them excellent candidates in biomedical application (Li *et al.,* 1997; Barbosa *et al.,* 2005; Vepari and Kaplan 2007). Therefore the desire to develop and create a new biomaterial for medical application using natural biodegradable polymer such as cellulose is always rational approach (Nair & Laurencin 2005; Barbosa *et al.,* 2005; Alriols *et al.,* 2008).

Cellulose is the world's most abundant natural occurring polymer which is produced by plants, as well as by microorganism. It is a linear homopolymer of glucose $(C_6H_{10}O_5)n$ that is insoluble in water and degradable in nature by microbe and fungal enzymes (Granja *et al.,* 2001b; Muller *et al.,* 2006). Cellulose is one of the natural polymer groups with the longest and widest medical applications experience. Non-toxicity (monomer residues are not hazardous to health), water solubility or high swelling ability by simple chemical modification, stability to temperature and pH variations made cellulose as being the widest use in medical applications (Fricain *et al.,* 2001; Granja *et al.,* 2001b,2005; Barbosa *et al.,* 2005; Nair &Laurencin, 2007).

In biomedical applications cellulose derivatives have been extensively investigated as dressings in treating surgical incisions, burns, wounds, hemodialysis membranes, coating materials for drugs, drug-releasing scaffolds, and various dermatological disorders (Entcheva *et al.,* 2004; Nair & Laurencin 2005; Granja *et al.,* 2005). Oxidized cellulose was used as a wound dressing and has been proposed for bone regeneration. Regenerated cellulose hydrogels (cellulose regenerated by the viscose process, CRV) have been investigated as implantable materials in

orthopaedic surgery, as sealing materials for the femoral component in hip prostheses, in place of the acrylic cement (Granja *et al.,* 2005).

Nevertheless, a full bioactive character cannot be attributed to normally occurring cellulose because of its lack of osteoinduction. Phosphorylation was therefore envisaged as the mean to enhance cellulose bioactivity. Once implanted, phosphorylated cellulose could promote the formation of calcium phosphate (which has a closer resemblance to bone functionality), hence ensuring a satisfactory bonding at the interface between hard tissue and biomaterial. The derivatives of pentavalent phosphorus such as H_3PO_4, P_2O_5, organic phosphate and $POCl_3$, were most frequently used to obtain phosphorus containing cellulose derivatives (Suflet *et al.,* 2006). Phosphorylated material based on cellulose regenerated by viscous process (CRV) were found to be non-cytotoxic in cultured human osteoblast as well as fibroblasts, having the ability to induce the formation of an appetite layer in simulated physiological condition (Granja *et al.,* 2005; Jayakumar *et al.,*2009).

This present study concerns the investigation of cellulose from oil palm biomass as potential raw material for the synthesis of CP for used as biomaterial. As a potential biomaterial, tissue compatibility has to be studied. For this purpose, CP synthesized will be exposed to mouse skin fibroblast (L929) and the cytotoxicity and proliferation accessed. Bioactivity of the material will be carried out by immersing the material in a simulated body fluid (SBF), where the ion concentration of the solution is approximately equal to human blood plasma, for 30 days.

1.2 Objectives

- To produce and characterize cellulose phosphate from oil palm empty fruit bunch.
- To investigate the biocompatibility of cellulose phosphate on connective mouse skin fibroblast (L929).
- To assess the bioactivity of cellulose phosphate in physiological environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil palm

The oil palm tree, (*Elaeis guineensis*), is one of the most important agriculture plants in Malaysia. It's originated from West Africa where it was growing and later developed into an agricultural crop (Law *et al.,* 2007). The oil palm is an erect monoecious plant that produced separate male and female inflorescences (Teoh, 2002). The oil palm trees are shown in Figure 2.1.

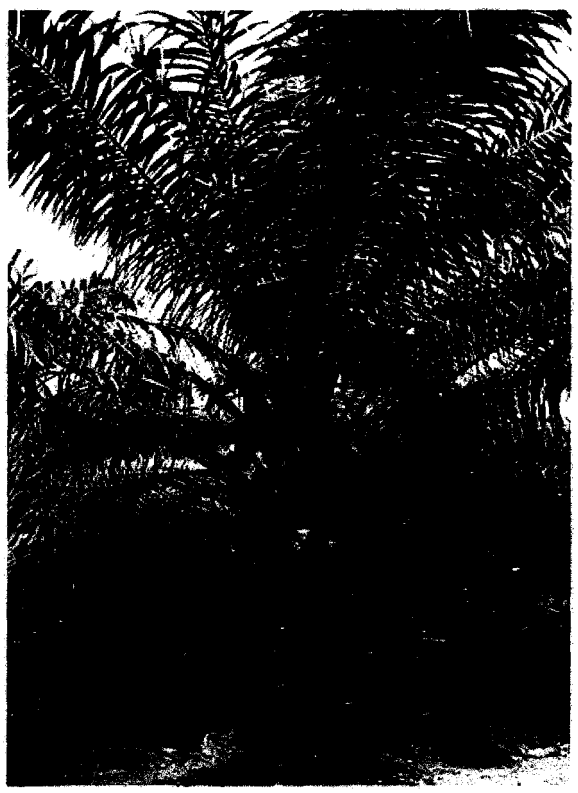

Figure 2.1: Oil palm tree

Climatic conditions which are hot and humid throughout the year make oil palm cultivation suitable in Malaysia. This view has been supported by Fitzherber et al. (2008) where climatic condition was important factor in growing oil palm, because oil palm need high-rainfall, low-lying areas, a zone naturally occupied by moist tropical forest, the most biologically diverse terrestrial ecosystem on Earth. Figure 2.2 showed the oil palm fresh fruit bunch and the structure of the palm fruit.

Botanically, oil palm is classified thusly:

Kingdom	: Plantae
Sub-Kingdom	: Tracheopionta
Division	: Angiospermae
Class	: Monocotyledones
Subclass	: Arecidae
Order	: Arecales
Family	: Arecaceae
Genus	: Elaeis

Figure 2.2: Oil palm fresh fruit bunch and the structure of the palm fruit

This plant was first introduced to Malaysia in early 1870's, as an ornamental plant, and in 1917 first commercial oil palm estate in Malaysia was set up at Tennamaran Estate, Selangor. The cultivation of oil palm rapidly increased beginning in the sixties under the government's agricultural diversification program which to reduce the country's economic where dependence on rubber and tin. Later in the 1960s, the government introduced land settlement schemes for planting oil palm as a means to eradicate poverty for the landless farmers and smallholders. The oil palm plantations in Malaysia are based on the estate management system and small holders' scheme (Mohamad *et al.,* 1985; MPOC, 2010).

In terms of hectare, the total area under oil palm cultivation is over 2.65 million hectares, which producing over 8 million tonnes of oil annually. The oil consists of only 10 % of the total biomass produced in the plantation. The remainder consists of huge amount of lignocellulosic materials such as oil palm fronds (OPF), oil palm trunks (OPT) and oil palm empty fruit bunch (OPEFB). The residues are Figure up as follows:

- 7.0 million Tonnes of oil palm trunks.
- 26.2 million Tonnes of oil palm fronds.
- 23 % of Empty Fruit Bunch (EFB) per tonne of Fresh Fruit Bunch (FFB) processed in oil palm mill (ASEAN, 2010).

2.1.1 Oil palm Industry in Malaysia

Malaysia oil palm industry started in 1917 and grew slowly until late 1950s due to switch over from rubber to oil palm during agricultural diversification policy. From the onward, the industry started to grow rapidly and currently very little room remains for any significant increase in oil palm plantations in peninsular Malaysia.

All future growth of oil palm is expected to be in Sabah and Sarawak (Abdul Khalil *et al.,* 2001).

Despite this enormous production, the oil is only a minor fraction of the total biomass produced in the plantation. The remainder consists of a huge amount of lignocellulosic material in form of OPF, OPT, and EFB. The year 1985 was considered as the start of a major replanting era in the oil palm industry, and from 1985 to 1995, there was a steady increase in oil palm replanting. By 1997, the replanting era reach maximum, yield over 27 million tonnes of biomass. As such, the oil palm industry must be prepared to take advantage of the situation and utilize the available biomass in the best possible manner (Rozman *et al.,* 2004; Yusof, 2007).

The status of oil palm biomass in Malaysia during 2006 as stated by Anis et al. (2007) showed that the total area of oil palm trees planted was 4.17milion hectares. Oil palm mills generally generate large amount of biomass waste. The amount of biomass produced by an oil palm tree included lignocellulosic material an average of 231.5 kg dry weight/year. In the year 2008, OPEFB and OPT are the major contributor of oil palm biomass, whereby about 15.8 and 8.2 million tonnes, respectively, have been produced annually (Sumathi *et al.,* 2008).

To date, 4.49 million hectares of land in Malaysia is under oil palm cultivation; producing 17.73 million tonnes of palm oil and 2.13 tonnes of palm kernel oil. Malaysia is one of the largest palm oil producers and exporters in the world, accounting for 11 % of the world's oils & fats production and 27 % of export trade of oils & fats (Abdul Khalil *et al.,* 2008; MPOC 2010). Figure 2.3 Comparison of Annual Production of Palm Oil between Malaysia and other producers.

Now palm oil industry has become an important source of income to the country. In Malaysia more than 17 million tons per year of palm oil crude produced and has been exported to over 150 countries, where China was a major importer, Table 2.1 summarised the annually oil palm export from Malaysia.

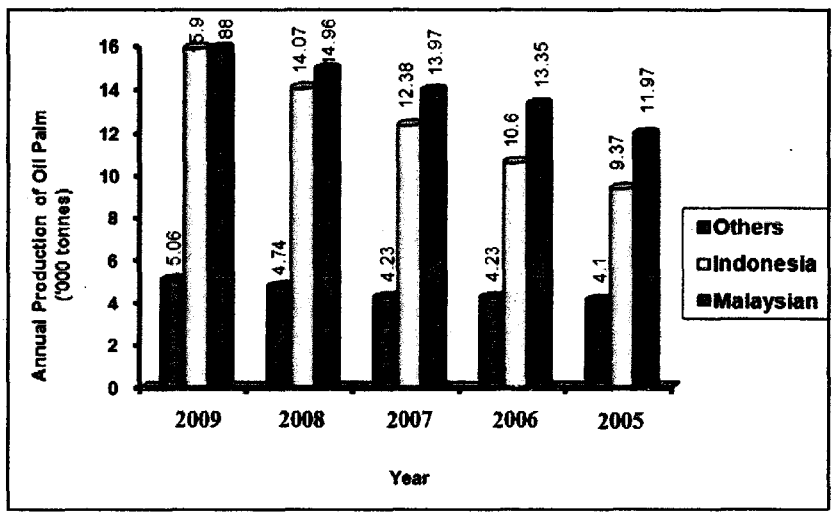

Figure 2.3: Annual production of palm oil between Malaysia and other producers (Source: MPOB, 2009).

Table 2.1: Exports of palm oil annually: 2004 – 2008

Year	Crude palm oil	Processed palm oil	Palm oil
2004	1,324,479	11,257,313	12,581,792
2005	1,611,621	11,833,890	13,445,511
2006	2,376,542	12,046,626	14,423,168
2007	1,935,927	11,810,895	13,746,823
2008	2,336,577	13,075,935	15,412,512

Source: MPOB, 2009

2.1.2 Oil palm lignocellulosic By-products

Besides crude palm and kernel oils, palm oil mill also generates massive amounts of lignicellulosic residues such as OPT, OPF during replanting and pruning, EFB and the mesocarp fibers during milling. These by-products, if not utilised and managed properly, will impose a disposal problem to the oil palm mill. In 1980, depending on the type of residue, oil palm residues is utilised as mulch, boiler fuel, or as fertiliser in the fields in order to reduced the environment impact paving the way toward a zero-waste policy (Abdul Khalil *et al.,* 2008).

The economic life span of the palm is about 25 years. However, the main economic criteria for felling (with the purpose for replanting) are the height of palm which reaches 13 m and above and annually yield of bunches falling below 10 to 12 tonne per hectare. This felling activity under the project of replanting produces a large quantity of lignocellulosic by product, in the form of stem, as well as frond (Sumathi *et al.,* 2008). The estimate of trunks and crown fronds at the time of replanting and from annual pruning from a hectare of oil palm are given in Table 2.2.

Table 2.2: Annual dry weight of trunk and fronds

	Dry weight/area (tonne/hectare)
Trunk at felling (OPT)	75.5 – 84.0
Frond at felling (OPF)	14.5 – 16.0
Annual pruning of fronds (OPF)	10.4 – 11.0

Source: Sumathi *et al.,* 2008

The dry weight of OPT and OPF per hectare area are varied due to the different number of palm planted per hectare. Sumathi et al. (2008) has pointed out that based on the mature hectarages, assuming that 3 percent of the palm oil in Malaysia is replanted annually. The annual estimated quantity of oil palm trunk and frond available from replanting are more than 6 million and 1.2 million tonne respectively. In addition, the dry weight of frond obtained during annual pruning and harvesting is estimated to be about 29.3 million tonnes.

Figure 2.4: EFB dumping waste at oil palm mills

Besides trunks, a large amount of fronds also generated during replanting. Indeed, the annual production of felled and pruned fronds exceeds from trunk. Currently, a certain amount of pruned fronds is used as interior mulching to conserve soil moisture in the plantations. However, this utilization has been found to attract rats, snakes, insect pests and thus limited its application. As a result a large quantity of lignocelluloses resource is still available for exploitation.

OPEFB are partly dried in the sun and later used as fuel, if not incinerated or applied to the fields. An economic use of OPEFB is to return them to the plantation as a mulch to enhance moisture retention and organic matter in the soil. On the other hand, the ash recovered from the incinerated OPEFB can also be sold or used as fertilizer in the palm plantations. The incineration of OPEFB emits particulates into the surrounding atmosphere and indiscriminate dumping of OPEFB causes additional methane emission into the atmosphere. The OPEFB, have traditionally been burnt and their ash recycled into the plantation as fertilizer. However, due to the pollution problem, incineration of EFB has been discouraged. Instead EFB is returned to the field to act as mulch (Nasrin *et al.,* 2008).

2.1.3 Oil Palm Empty Fruit Bunch (OPEFB)

OPEFB is one of such solid waste generated in oil palm planted throughout Malaysia over 4 million hectares land, which remains unutilized commercially. Hence, it has been verified in engineering properties and construction products made from OPEFB satisfy the requirement of consumers' trend using products made from eco-friendly and natural waste.

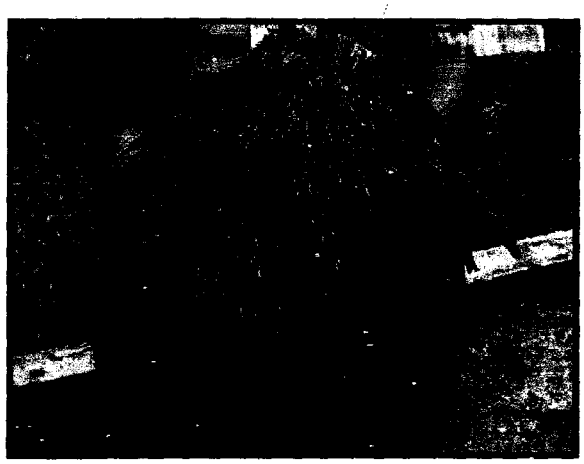

Figure 2.5: Oil palm empty fruit bunches

As a source of both edible and industrial oils, oil palm is one of the most important tree species in Southeast Asian countries like Malaysia. However, woody fibrous residues, which remain after oil is extracted, have not been effectively utilized so far. These so-called EFB (Figure 2.5) could serve as an alternative source for cellulose pulp, or dissolving pulp, (DP) which could be used as a raw material of cellulose derivatives and regenerated cellulose (WanRosli *et al.,* 1998, 2003, 2004).

2.1.4 Application of OPEFB Biomass.

Recently, utilization of biomass resources has been the subject of various studies. Among the oil palm fiber residues, OPEFB offer the best prospect for commercialize since its ready available at the palm oil mill, which can minimize transportation and procurement cost. In Malaysia, OPEFB is one of the biomass materials, which is a by-product from the palm oil industry. The production of OPEFB was around 5.3 million per year (Rozman *et al.,* 2004; Myrtha *et al.,* 2008).

OPEFB has been investigated as a raw material for building materials and are now used for other value-added products like pulp, medium density board, wood composite product and fiberboard. In general, utilization of biomass in lignocellulosic composite has been attributed to several advantages such as having low density, greater deformability, less abrasiveness to equipment, biodegradable and low cost (Rozman *et al.,* 2004).

Through the initial review is believed to be a profitable method in producing innovative products, from OPEFB. Now varieties products produced from OPEFB, such food packaging, pulp, fertilizer and medium density fiberboard and for energy purpose such as ethanol, and biodiesel bio-methane (Yokoyama and Matsumuya, 2008). OPEFB contains high cellulose, lignin and hemicelluloses. The chemical

composition (Table 2.3) showed that OPEFB have a good potential usage in various industries where cellulose as raw material in producing product.

Table 2.3: Chemical composition of OPEFB

Composition	% Dry EFB
Holocelulose	82.4±1.4
Cellulose	62.9 ± 2.0
Hemicellulose	28.0
Lignin (non dissolved in acid)	18.8 ± 0.3
Free Ash Lignin (non dissolved in acid)	17.8 ± 0.02
Dissolved Material 1% NaOH	14.5 ± 2.7
Material Dissolved in Hot Water	7.5 ± 0.8
Extractive	3.7 ± 0.3
ASH	1.3 ± 0.2

Source: Law *et al.*, 2007

As example product that successfully produced from OPEFB cellulose-based is Palmcell ® which is carboxylmethylcellulose (CMC). This material has the potential to be used in paper manufacturing process and as additives to improve paper strength. As a result the strength of paper produced increase either in the dry and wet conditions. The products not only efficient to increase the strength of the paper, but the cost for production Palmcell ® are relatively low compared to other

additives. This is because the use of OPEFB as a main ingredient in the manufacturing process.

Law et al. (2007) has made a study of some fiber derived from oil palm tree, and concluded the dissolved pulp derived from EFB are suitable as raw materials for produce various grades of paper. Continuing research and study has increased the diversity of applications of OPEFB as an alternative source. For example filling mattresses and cushions, footwear and automotive hardware already used OPEFB as raw material. OPEFB products applied in the manufacturing process has now been successfully commercialized and exported. Looking to the efforts done, OPEFB could be important source of biomass alternative in many industries.

2.2 Biodegradable Polymer

Over the last few decades, the development of biocompatible and biodegradable materials also known as biomaterials used for biomedical applications have been made (Nair and Laurencin, 2005). These materials were intended to replace part of a living system or to function closely with living tissue. The requirements for use in medical applications, the biodegradable polymer must be biocompatible and biodegradable (Correlo *et at.,* 2008; Liuyun, *et al.,* 2009; Salmoria *et al.,* 2009).

The term biocompatibility was referred to the ability of biomaterial to provoke an acceptable cellular and biological response from the host environment, and in a clinical context this entails not producing a toxic or injurious reaction and not causing immunological rejection (Matthew, 2002; Vepari *et al.,* 2007; Correlo *et al.,* 2008).

On the other hand, the term biodegradable polymers refer to material that can degrade *in vitro* and *in vivo*, either into products that are normal metabolites of the body or into products that can be completely eliminated from the body with or without further metabolic transformations (Dumitriu, 2002; Nair and Laurencin, 2005).

Several classes of inorganic materials, such as metals, ceramics and glasses also polymeric material either natural or synthetic have been approved for medical used (Nair and Laurencin, 2005; Correlo *et al.,* 2008). Some of synthetic material those physical properties most similar to natural tissue was successful used for tissue replacement (Bhat, 2005).

Metal because of their excellent electrical and thermal conductivity and also good mechanical properties, made it suitable being biomaterial, three main alloys, which is titanium-aluminium, stainless steel and cobalt-chromium alloys were used for the high load bearing application in skeletal system. Conducting metal like platinum and platinum-iridium alloys was used for electrical stimulation heart, also for muscles and nervous tissue. In orthodontics application nitinol an alloy of nickel and titanium were already used (Peng *et al.,* 2006; Bedi *et al.,* 2009).

Ceramic defined as the art and science of making and using solid articles that have essential inorganic non-metallic material components (Bhat, 2005; Habraken, *et al.,* 2007). Alumina which used in orthopaedic joint replacement is extremely stable and inert ceramic material. The chemical inertness and high abrasive resistance provide improvements over the widely used metal. In improving the surface properties of alumina and metal alloys bio-glass was employed. The degradable ceramics, which are almost invariably used on calcium phosphates, find application in hard tissue regeneration. In cardiovascular application, inert carbons are used to

improve blood compatibility and also find in dental applications (Kokubo *et al.*, 1992; Habraken *et al.*, 2007).

Particles and fibers reinforcement has been used to improve properties of biomaterials, for example barium sulphate particles in Poly(methylmethacrylate) (PMMA) cement improves stiffness and fatigue life considerably. In improving the strength for rubber which used in catheters very fine particles of silica (SiO_2) was usually reinforced. In modern dental composite materials were blend of glass or ceramic particles dispersed in polymeric organic resin matrix with interfacing saline coupling agents. The composite such as graphite fibers in epoxy resin can be strong as steel but much lighter. However, this material is complaint when the loaded transversely to the fibers (Marques *et al.*, 2009).

A number of synthetic polymers find application as biomaterial. They include polyolefins, polyamide, polyesters, polyurethanes, polyacylates, polysulfone, polyethers, and silicon rubbers. Some of these materials are also used as sutures, tissue adhesives, shunts, catheters and space fillers. Biodegradable polymers, such as natural, synthetic polyesters, and polyamides were employed as bone plates which provide temporary scaffolding or support respectively, while natural tissue regeneration take place (Chandra and Rustgi, 1998; Nair and Laurencin, 2007).

Among them, polymeric materials are widely used in clinical applications because of their unique physical and chemical properties. According Bhat, (2002) polymers have physical properties that almost close to soft tissue and this class of material were used to replace the function of soft tissue including skin, tendon, cartilage, vessel walls, lens, breast and bladder. On the other hand because of its ability to be structurally and functionally modified, also their biodegradation easy to control and because they are relatively inexpensive to manufacture made polymeric

material as candidates for various medical applications (Barbosa *et al.,* 2005; Correlo *et al.,*2008; Salmoria *et al.,* 2009; Liuyun *et al.,* 2009).

2.3 Natural Polymer

The used of structural material from natural polymer or biopolymer is not a new phenomena. Materials derived from animals or plants world already used as biomaterials. Due to similarity of this material with material to human bodies gives natural polymer huge advantage (Barbosa *et al.,* 2005; Jayakumar *et al.,* 2009). The natural polymer materials do not offer the problems of toxicity that often faced by synthetic material. Some of natural material may carry specific protein binding sites and other biochemical signals that may assist in tissue healing or integration. One of the examples of a natural material was collagen, which existed mostly in fibril form, has a characteristic triple-helix structure and the most prevalent protein in the animal world (Jayakumar *et al.,* 2009).

Other natural materials under consideration include coral, chitin from insects and crustaceans, keratin from hair and various natural biodegradable polymers such as polysaccharides which is currently being investigated as matrices for controlled drug delivery or as scaffolds for tissue engineering (Nair and Laurencin, 2005; Barud, 2007).

Due to their excellent biocompatibility, natural biodegradable polymers seem to be the choice for biomedical applications. This is because they closely mimic native cellular environments, have unique mechanical properties, and are biodegradable by an enzymatic or hydrolytic mechanism (Granja *et al.,* 2005; Barbosa *et al.,* 2005; Nair and Laurencin, 2005; Wan *et al.,* 2006).

According to Chu, (2002), biodegradable polymer do not elicit permanent chronic foreign body reaction due to the fact that they would be gradually absorbed by human body, and also they didn't permanently retain trace of residual in the implantation sites. Furthermore some of them have been found to be able to regenerate tissues, through the interaction of their biodegradation with immunologic cells like macrophages (Li *et al.,* 1997; Bhat, 2002).

Advantages of biodegradable polymers are once implanted they no need for a second surgical procedures as well as eliminate the long-term biocompatibility concern. The biodegradation may offer other advantages in many short-term medical applications; in orthopaedic applications mechanically incompatible implants such as metallic implants can sometimes lead to stress shielding, whereas biodegradable implants may slowly transform the load as it degrades (Li *et al.,* 1997; Nair and Laurencin, 2005).

Natural biodegradable polymers remain attractive because they are economical, readily available, capable of chemical modifications, and potentially degradable and compatible due to their origin. In addition degradation of natural biodegradable polymers into physiological metabolites makes them excellent candidates for a wide range of applications, such as drug delivery (Satturwar *et al.,* 2003).

However, natural biodegradable polymers not been fully exploited in the biomedical field, this is due to the inherent disadvantages such as risk of viral infection, antigenicity, unstable material supply, and batch-to-batch variation in properties. Another problem faced by these materials, is their tendency to denature or decompose at temperatures below their melting points. This severely limits their

fabrication into implants of different sizes and shapes (Stillwell *et al.*, 1997; Nair and Laurencin, 2005).

2.3.1 Polysaccharides

Polysaccharides are high molecular weight polymers, which is having one or more monosaccharide repeating units (Figure. 2.6). Polysaccharides, have excellent properties such as non-toxicity (monomer residues are not hazardous to health),water solubility or high swelling ability by simple chemical modification, stability to pH variations, and a broad variety of chemical structures which make these group of polymer the longest and widest experience in medical applications (Nair and Laurencin, 2005). All of these advantages able to overcome some of its disadvantages such as low mechanical properties, temperature and chemical stability, and enzymatic degradation (Barbosa *et al.*, 2005).

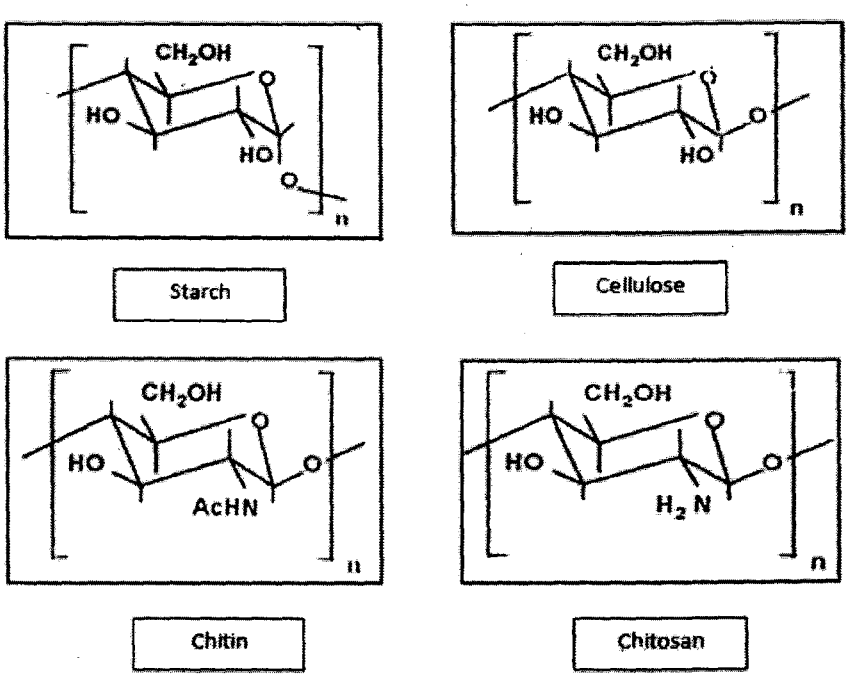

Figure 2.6: Structure of polysaccharides. Source: Chandra and Rustgi, 1998.

Furthermore with wide availability, cost effectiveness, easily modified with the presence of reactive functional groups along the polymer chain, biodegradability, biocompatibility, and water solubility, combined with the ability to form hydrogels, make them excellent candidates for tissue engineering and drug delivery applications (Nair and Laurencin, 2005,2007; Bhat, 2002; Muller *et al.*, 2006).

2.3.1.1 Cellulose

Cellulose is the main component of plant cell walls and one of the most abundant, renewable and biodegradable organic compounds on earth. In wood contents about 40-50 % cellulose, 98 % of cellulose was in cotton fibers, 70 % in bast fiber like hem and located predominantly in the secondary wall (Figure. 2.7). Cellulose can be produced from various other plant fibers, such as corn cobs or bagasse (sugar cane stalks), rice hulls, bamboo, and fibers such as EFB, kenaf, flax, pineapple leaf etc (Kamide, 2005). The used of cellulose in industry can be considered spacious, pulp and paper was the largest industries that used cellulose as raw materials in production process.

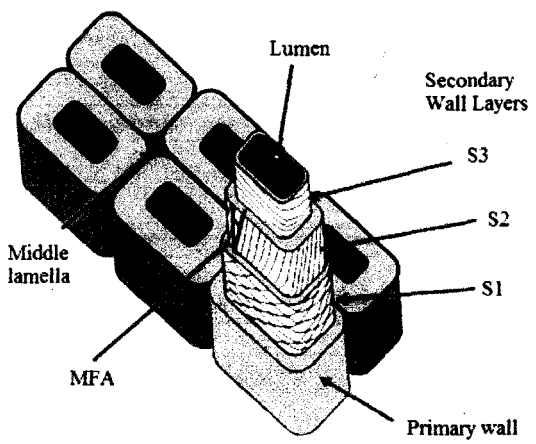

Figure 2.7: Structure of wood cell showed middle lamella (ML), primer wall (P), second wall layer (S_1, S_2, &S_3) and lumen. Source: David, 2003.

2.3.1.3.1 Cellulose chemistry

Cellulose is a white fibrous material composed of the elements Carbon (C), Hydrogen (H) and Oxygen (O_2). The chemical formula for cellulose is $(C_6H_{12}O_5)_n$, where n is the degree of polymerization (DP) or the number of repeating glucose monomer per cellulose chain with the average between 700 to 10000. DP of cellulose is different depending on the source and the process that cellulose gone through (Entcheva *et al.,* 2004). Cellulose applications are depending on DP value own by cellulose. Table 2.4 showed the different characterization of cellulose from source, molecular weight, and degree of polymerization.

Table 2.4: Molecular weight and degree of polymerization for cellulose from different source

Source	**Molecular weight**	**DP**
Pure Cellulose	600 000 - 1 500 000	3 500 - 10 000
Cotton	80 000 - 500 000	500 - 3 000
Wood Pulp	80 000 - 340 000	500 - 2 100
Filaments rayon	57 000 - 73 000	350 - 450

Source: Nissan *et al.,* 1965

Cellulose is a linear polymer with repeating units consisting of d-glucose in $^4C_1$ conformation (Figure. 2.8). It forms the structural framework of plants and was isolated in the form of micro fibrils. Hydrogen bonds either inter and intra (Figure. 2.9), made cellulose molecule interact with one and other (Entcheva *et al.,* 2004; Barbosa *et al.,* 2005; Granja *et al.,* 2005; Barud *et al.,* 2007).

The intra-molecule hydrogen bond, limit the movement of glucopyranose unit which is around acetyl bond (glycoside). However (1→4)-β bond need 180° rotation for single glucose unit for fulfil the β configuration which attached to hemiacetyl bond. Therefore cellulose molecule gives a stiff and rigid linear chain like rod (Nissan *et al.*, 1965).

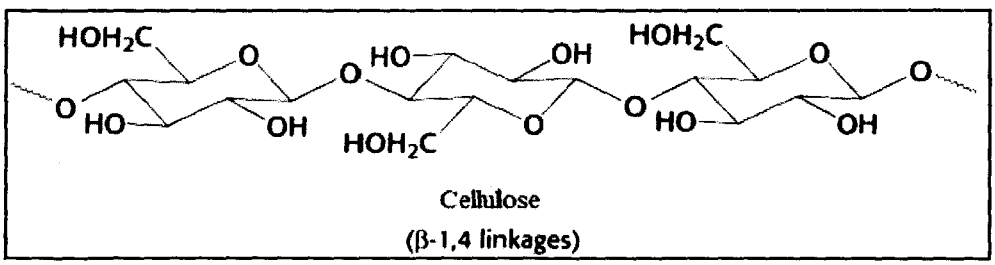

Figure 2.8: The cellulose molecule – $(C_6 H_{10} O_5)$ *n* configuration.

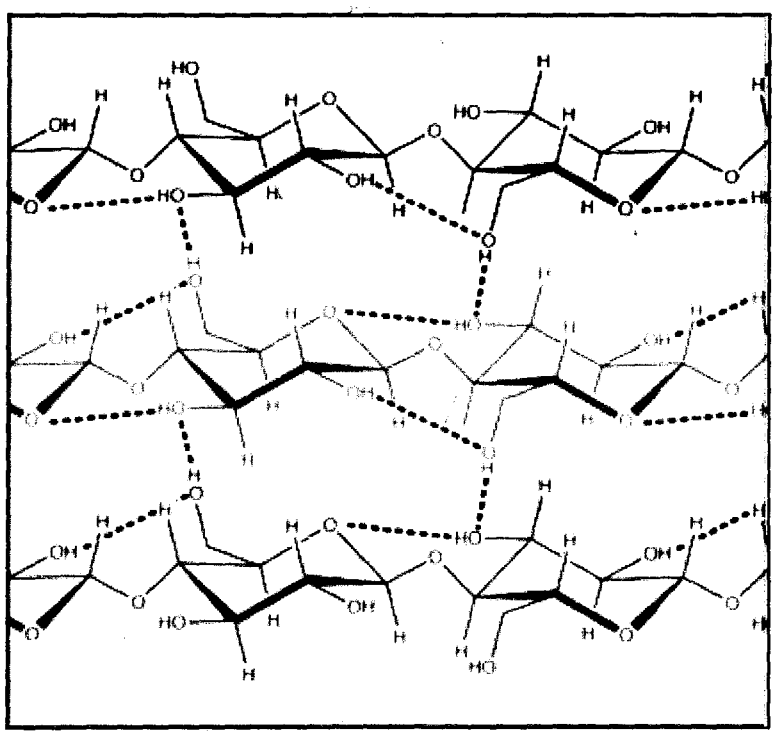

Figure 2.9: Cellulose chain (chair comformation). Dash line connecting between cellulose ring showed the intra and inter molecular bond.